

## Supplementary Information

### **Sulforaphane exhibits antiviral activity against pandemic SARS-CoV-2 and seasonal HCoV-OC43 coronaviruses in vitro and in mice**

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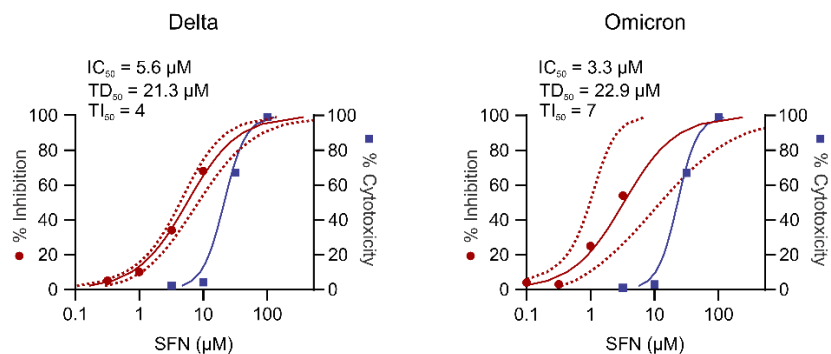
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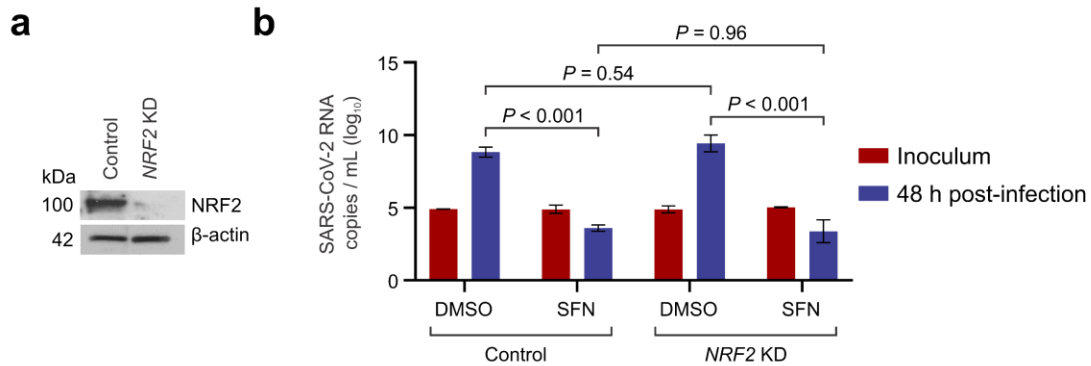
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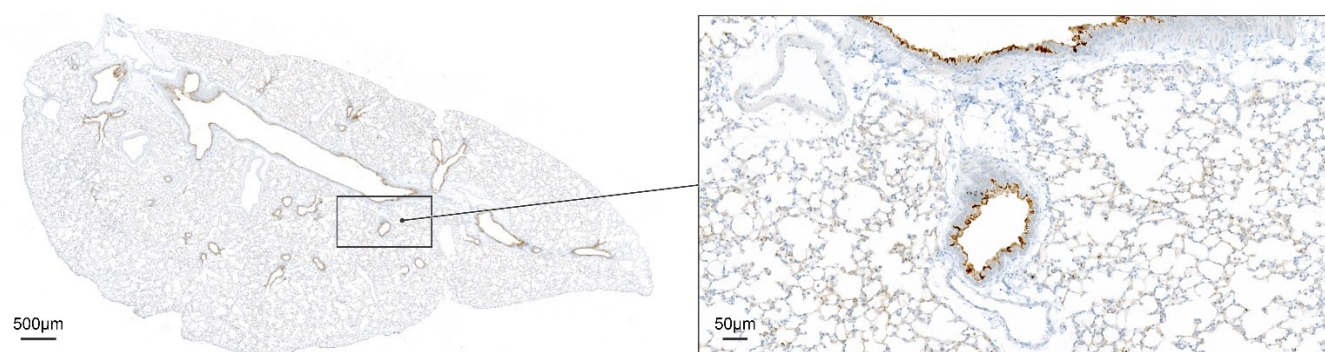
**Supplementary Figure 1. Antiviral effects of SFN against SARS-CoV-2 variants.** Effects of SFN evaluated in Vero C1008 cells exposed to drug for 1 hour followed by viral inoculation. Delta and Omicron variants of SARS-CoV-2 were evaluated for CPE using a bioluminescence readout. Antiviral data is displayed in red; anti-host cell activity (cytotoxicity) is displayed in blue.



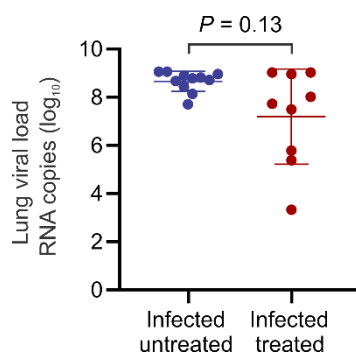
**Supplementary Figure 2. Antiviral activity of SFN on *NRF2* knockdown (KD) cells.** *NRF2* was silenced from Caco-2 cells by CRISPR/Cas9 to reduce the expression of *NRF2*. **(a)** Western blot determination of *NRF2*, in total cell lysate of Caco-2 cells, control, and *NRF2* KD, treated as described above.  $\beta$ -actin was used as loading control. **(b)** Control and *NRF2* KD cells were treated with SFN (5  $\mu$ M) or DMSO (vehicle control) over 1 - 2 h. Subsequently, the cells were infected with SARS-CoV-2/USA-WA1/2020 and incubated over 48 h. Culture supernatants were collected and processed for quantification of SARS-CoV-2 copies by qRT-PCR. A significant reduction in viral load was observed in cells treated with SFN in both control and *NRF2* KD cells ( $P < 0.001$ ). There was no significant difference in the viral load with or without SFN treatment in *NRF2* KD cells compared to control cells ( $P \geq 0.54$ ). Data representative of three independent experiments. Statistical comparisons were made with one-way ANOVA with Tukey's multiple comparisons test.



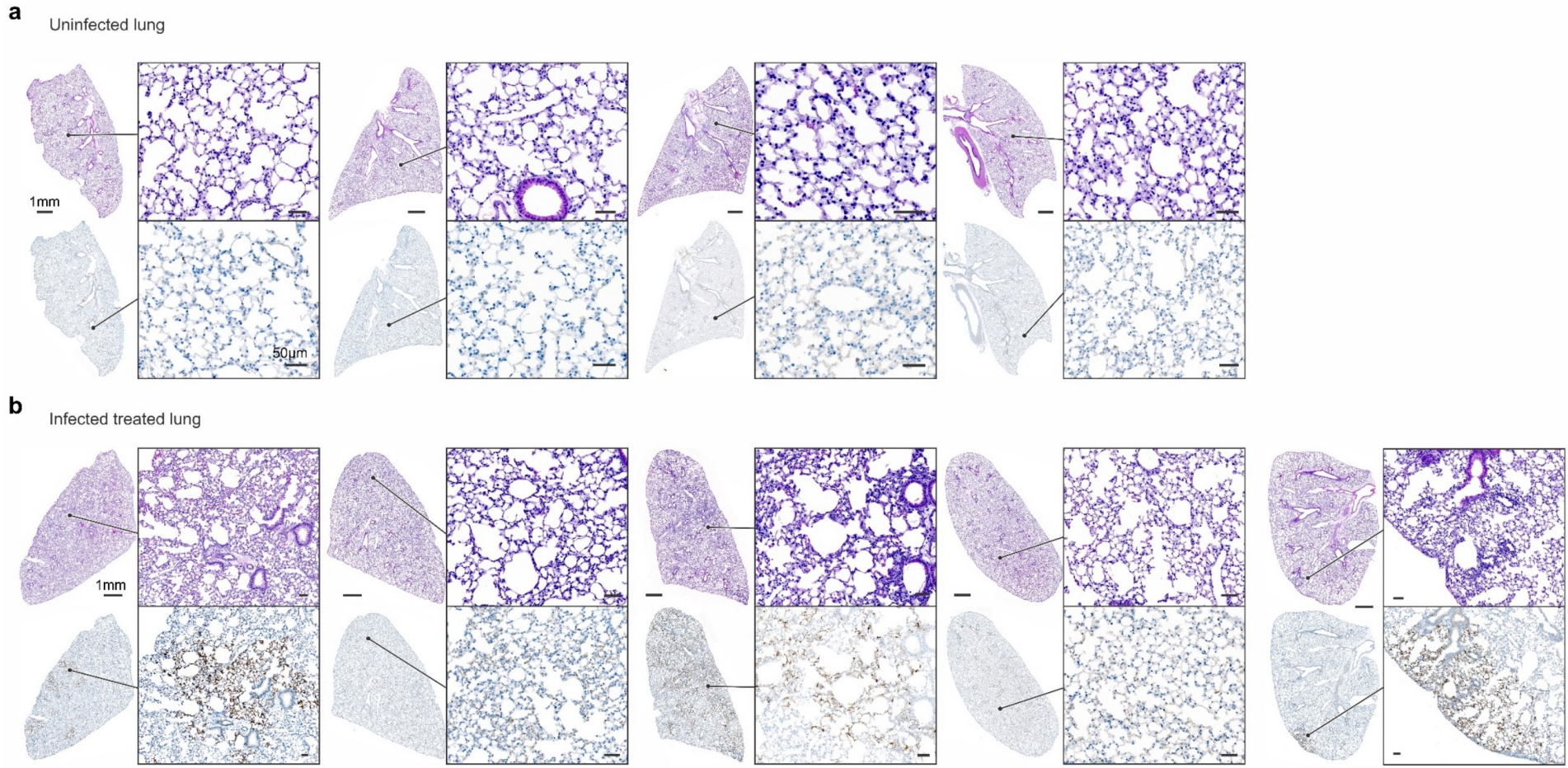
**Supplementary Figure 3. hACE2 expression in lung tissues.** The expression of hACE2 in the lung of K18-hACE2 mice infected with SARS-CoV-2 and treated with SFN was primarily in the airway epithelia. The lung section of representative mouse is shown.



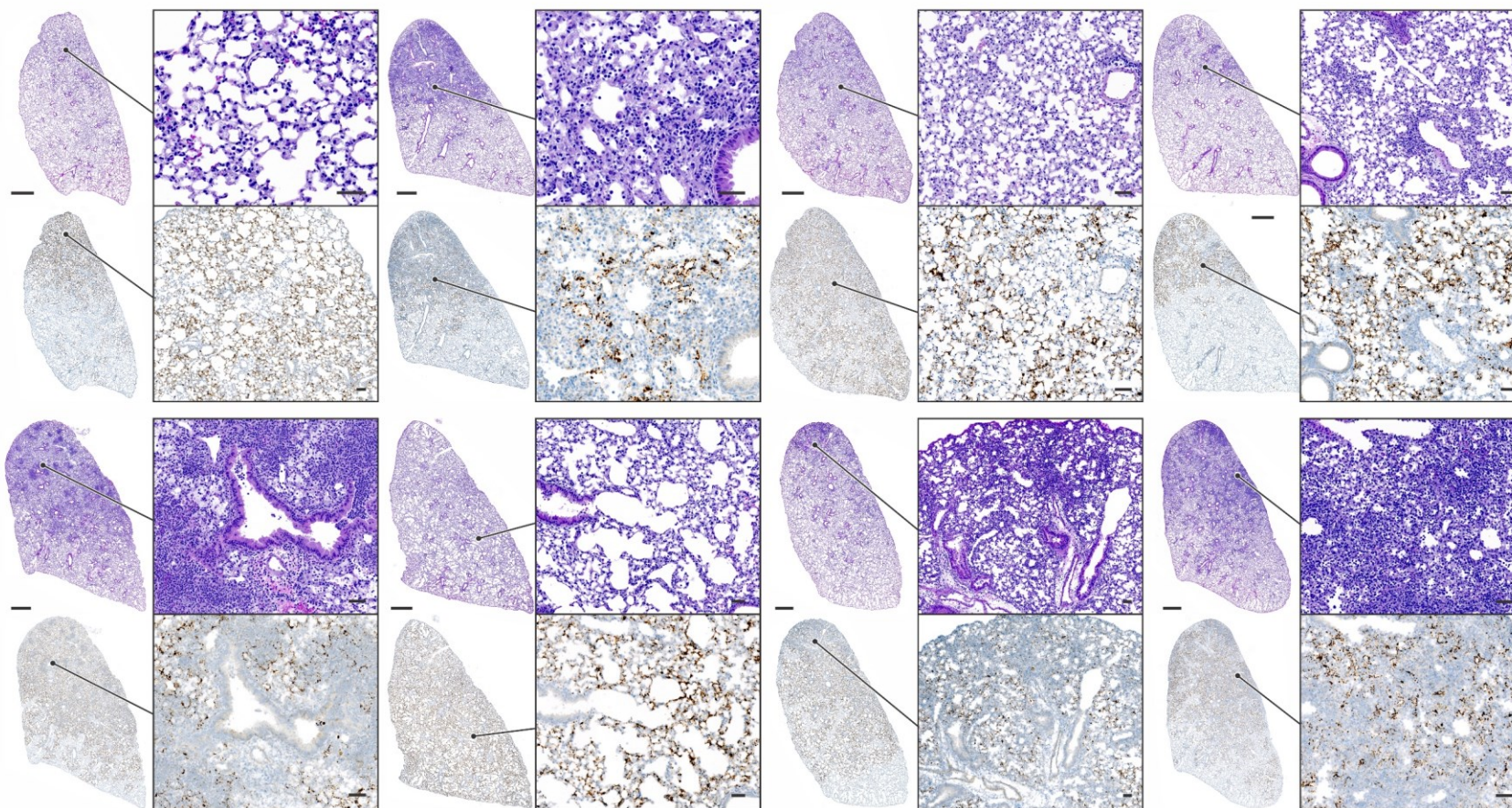
**Supplementary Figure 4. Pulmonary viral burden.** The viral load in the lungs of infected treated animals, represented as the total SARS-CoV-2 N protein copies, had a 1.46 log<sub>10</sub> reduction compared to infected untreated controls (Mann-Whitney *U* test, two-tailed, *P*=0.1308). Data not normalized to *Pol2Ra*. Data from two independent experiments, infected untreated (n=11), infected treated (n=9).



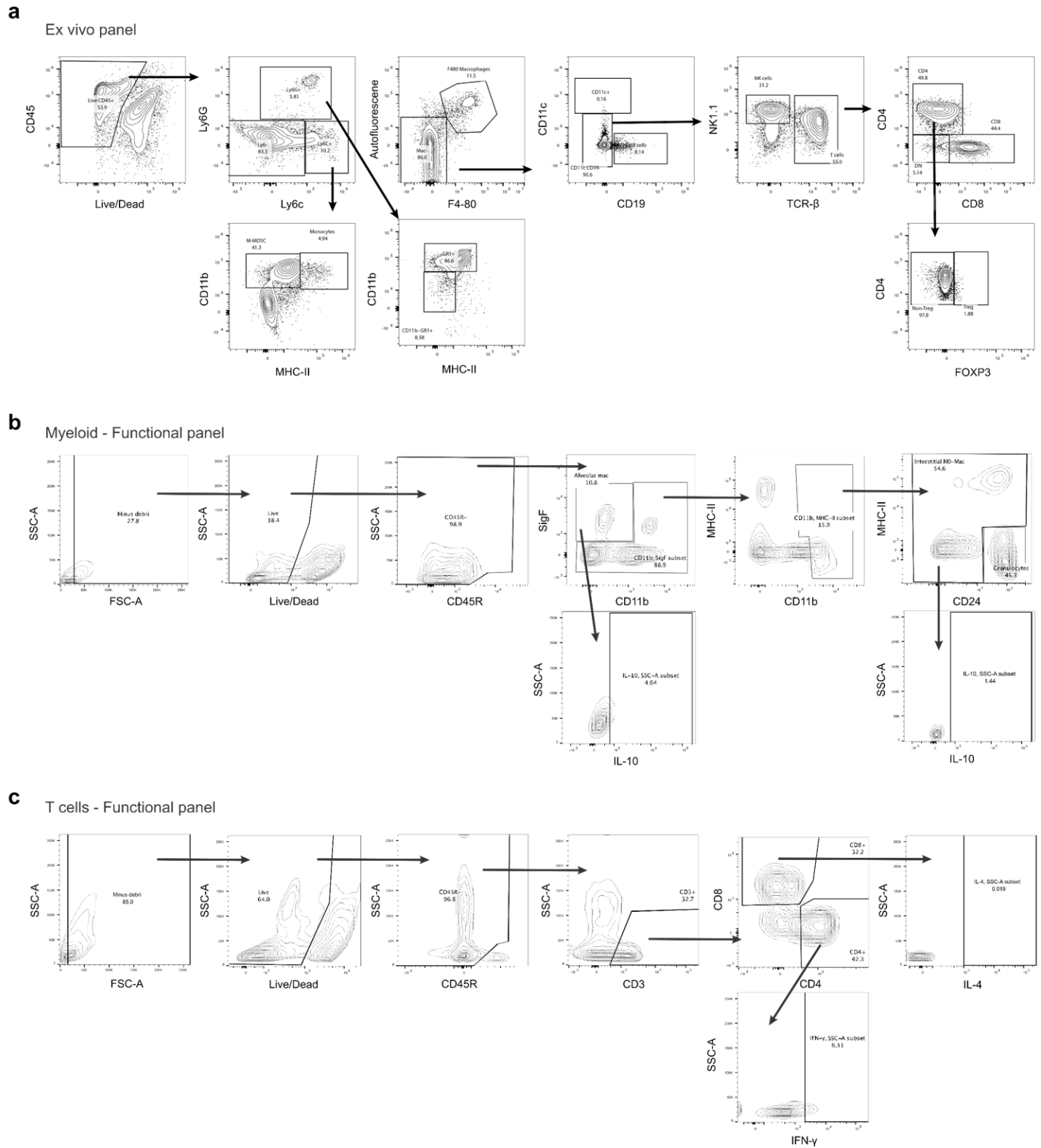
**Supplementary Figure 5. Histology and SARS-CoV-2 spike protein immunostaining.** Low magnification (scale bar, 1 mm) and the corresponding high magnification areas (scale bar, 50  $\mu$ m) of Hematoxylin and eosin staining (top panels) and SARS-CoV-2 spike protein immunostaining (bottom panels) of the lungs of (a) uninfected, (b) infected SFN-treated, and (c) infected untreated animals.



**C** Infected untreated lung



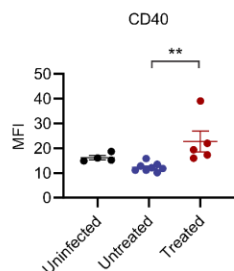
**Supplementary Figure 6. Flow cytometric gating strategy.** Flow cytometric gating strategy for macrophages (a), T cells (b), and T cell functional analysis (c), in lung, spleen, and bronchoalveolar lavage.



**Supplementary Figure 7. Functional markers of immune response in the lung.** Flow cytometric analysis of pulmonary alveolar macrophages (a) and interstitial macrophages (b). MFI, mean fluorescent intensity. Data represented as mean  $\pm$  standard error of mean. n=4 uninfected, n=5 infected SFN-treated, and n=8 infected untreated animals. Statistical comparisons made with one-way ANOVA, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

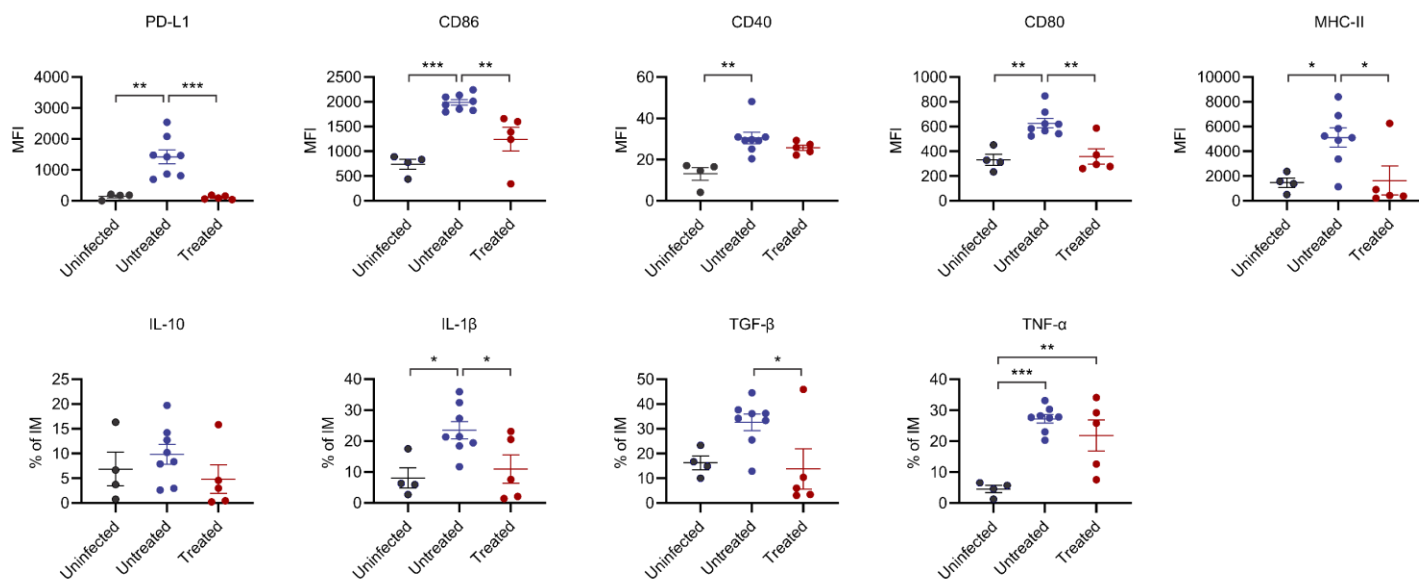
**a**

Lung - alveolar macrophages (AM)



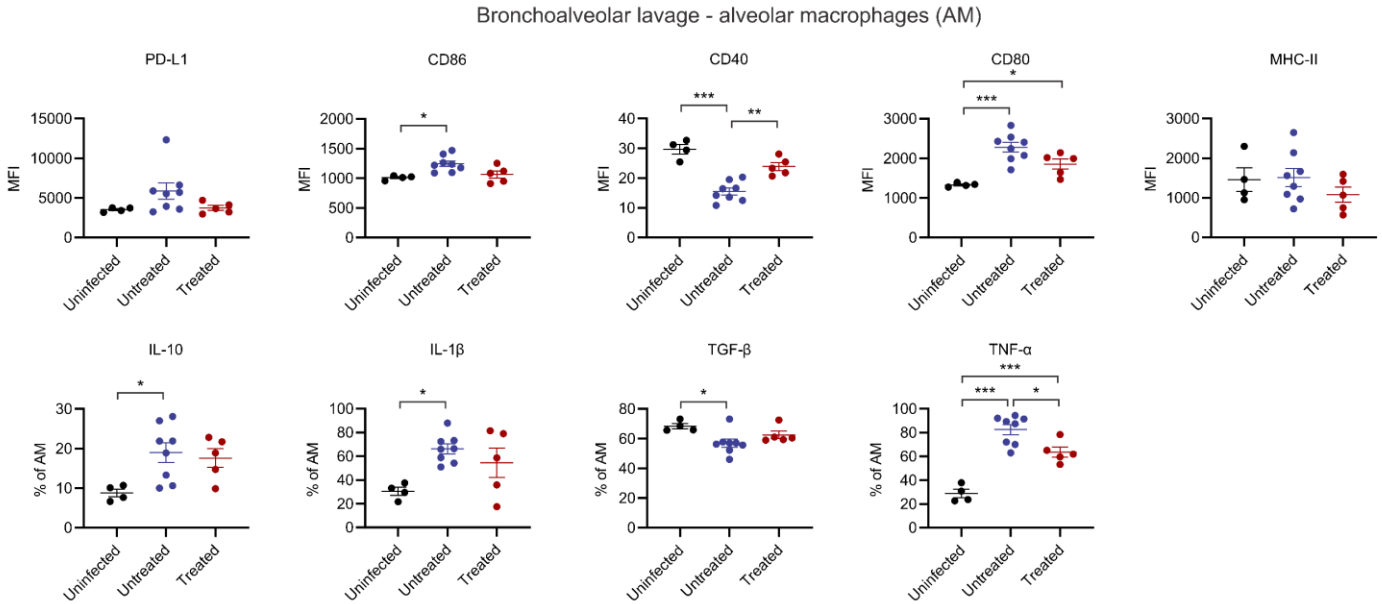
**b**

Lung - interstitial macrophages (IM)

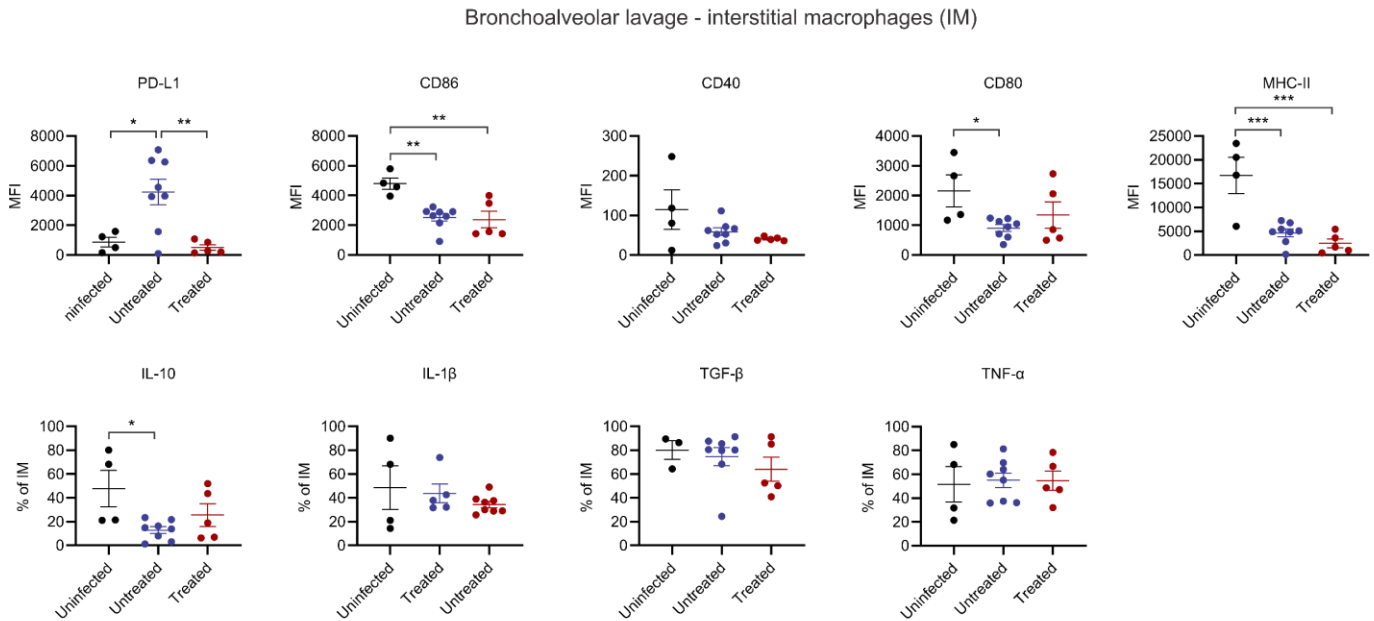


**Supplementary Figure 8. Functional markers of macrophages in the bronchoalveolar lavage.** Flow cytometric analysis of alveolar macrophages (a) and interstitial macrophages (b) of the bronchoalveolar lavage. MFI, mean fluorescent intensity. Data represented as mean  $\pm$  standard error of mean. n=4 uninfected, n=5 infected SFN-treated, and n=8 infected untreated animals. Statistical comparisons made with one-way ANOVA, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

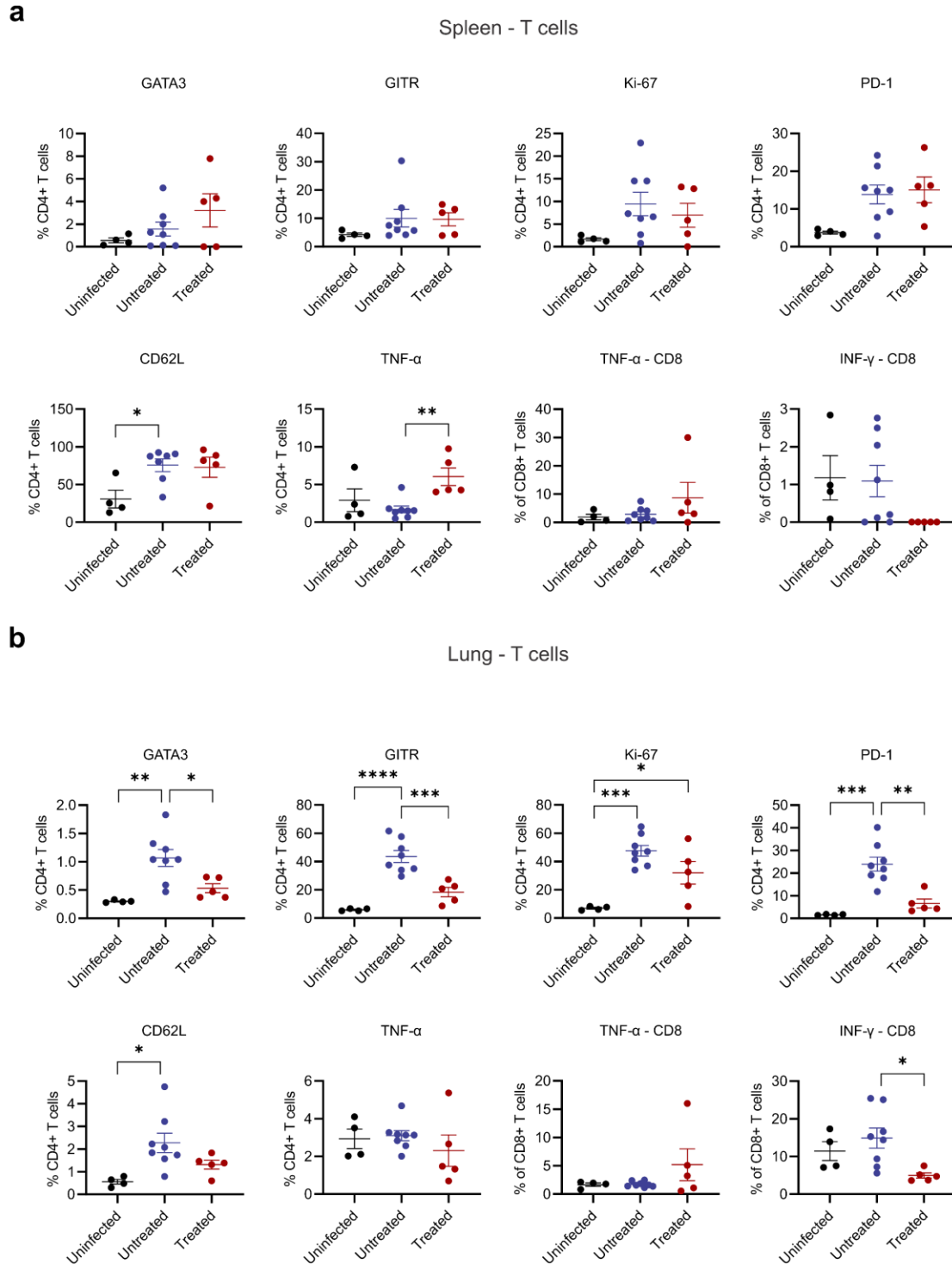
**a**



**b**

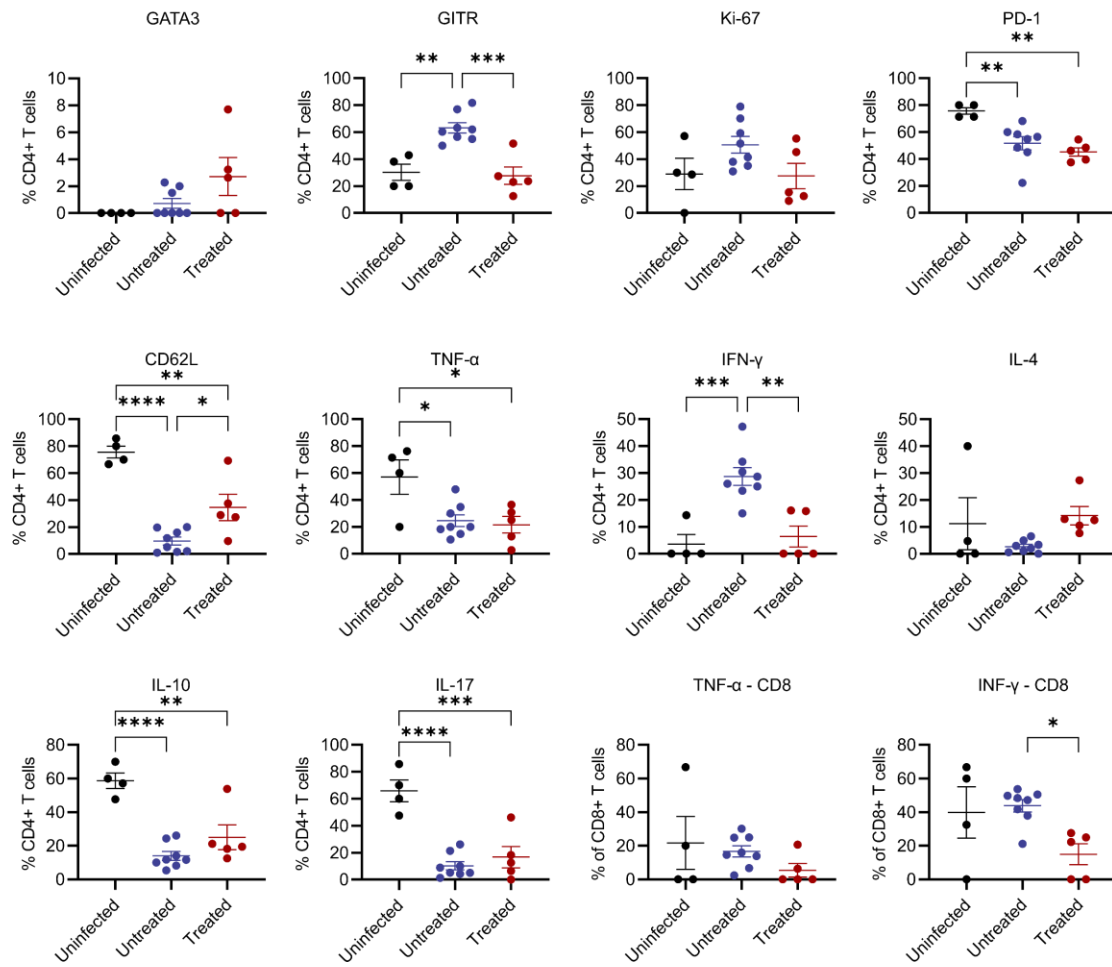


**Supplementary Figure 9. Functional characterization of T cells.** Flow cytometric analysis of T cells after stimulation with PMA/ionomycin in the spleen (a), lung (b), and bronchoalveolar lavage (c). MFI, mean fluorescent intensity. Data represented as mean  $\pm$  standard error of mean. n=4 uninfected, n=5 infected SFN-treated, and n=8 infected untreated animals. Statistical comparisons made with one-way ANOVA, \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001, \*\*\*\* $P$ <0.0001.



**C**

## Bronchoalveolar lavage - T cells



**Supplementary Table 1. Antiviral effects of SFN against HCoV-OC43.**

Panel	Cell line	Drug <sup>a</sup> (range tested, $\mu$ M)	Time of drug addition	Time of virus inoculation	IC <sub>50</sub> <sup>b</sup> ( $\mu$ M)	TD <sub>50</sub> <sup>b</sup> ( $\mu$ M)	TI <sup>b</sup>
A	Vero C1008	SFN (320 – 0.032)	1 – 2 h before virus	1 - 2 h after drug	10	73	7
B	MRC-5	SFN (320 – 0.032)	1 – 2 h before virus	1 - 2 h after drug	18	83	5
C	Vero C1008	SFN (320 – 0.032)	24 h after virus	24 h before drug	18	88	5
D	Vero C1008	SFN (100 – 0.01)	24 h before virus	24 h after drug: drug washed out and then virus added	21	91	4
E	Vero C1008	RDV (100 – 0.01)	1 – 2 h before virus	1 - 2 h after drug	22	142	6
F	Vero C1008	SFN (100 – 0.01) L to R <sup>c</sup> RDV (32 – 0.032) T to B	1 – 2 h before virus	1 - 2 h after drug	N/A <sup>d</sup>	N/A <sup>d</sup>	N/A <sup>d</sup>

<sup>a</sup> SFN, Sulforaphane; RDV, Remdesivir

<sup>b</sup> IC<sub>50</sub>, Median inhibitory concentration; TD<sub>50</sub>, Median cytotoxic dose; TI, Therapeutic index

<sup>c</sup> SFN diluted across plate, Left to Right (L to R); RDV diluted down plate, Top to Bottom (T to B)

<sup>d</sup> N/A, Not applicable. See Figure 1 and Results for Combination Index (CI) results

**Supplementary Table 2. List of evaluated cell lines.**

<b>Cell line*</b>	<b>Source (Catalog number)</b>	<b>Organism</b>	<b>Tissue</b>	<b>Morphology</b>	<b>Disease</b>
HCT-8 [HRT-18]	ATCC# (CCL-244)	Human	Colon	Epithelial	Adenocarcinoma
Caco-2	ATCC (HTB-37)	Human	Colon	Epithelial	Adenocarcinoma
MRC-5	ATCC (CCL-171)	Human	Lung	Fibroblast	Normal
Vero C1008 [Vero 76, clone E6, Vero E6]	ATCC (CRL-1586)	African green monkey	Kidney	Epithelial	Normal

\*None of the cell lines listed are registered as a misidentified cell line according to the International Cell Line Authentication Committee (ICLAC) Register of Misidentified Cell Lines, version 11. <https://iclac.org/databases/cross-contaminations/>

#ATCC, American Type Culture Collection.

**Supplementary Table 3.** List of antibodies used for flow cytometry.

Marker	Fluorophore	Vendor	Catalog #	Clone
<b>Ex vivo panel</b>				
NK1.1	PE CF594	BD Biosciences	562864	PK136
CD19	PE Cy5	BioLegend	115510	6D5
CD62L	PECy7	BD Biosciences	560516	MEL-14
CD11b	AF700	BioLegend	101222	M1/70
CD4	APC Cy7	BD Biosciences	565650	RM4-5
Ly6G	efluor 450	ThermoFisher	48-5931-82	RB6-8C5
CD103	BV480	BD Biosciences	566201	M290
CD44	BV510	BioLegend	103044	IM7
Ly6c	BV570	BioLegend	128030	HK1.4
PD1	BV605	BioLegend	135220	29F.1A12
TCRb	BV650	BioLegend	109251	H57-597
MCHII	BV711	BioLegend	107643	M5/114.15.2
CD11c	BV750	BioLegend	117357	N418
F4/80	BV785	BioLegend	123141	BM8
CD69	BUV737	BD Biosciences	612793	H1.2F
CD8	BUV805	BD Biosciences	612898	53-6.7
CD45	Super Bright 436	Fischer Scientific	62045182	30-F11
CPT1a	AF488	Abcam	ab171449	8F6AE9
VDAC1	AF532	Abcam	ab14734	20B12AF2
H3K27Me3	PE	CST	40724	C36B11
FOXP3	PerCpCy5.5	ThermoFisher	45-5773-82	FJK-16x
Ki67	PerCp-eFluor710	ThermoFisher	46-5698-82	SolA15
GLUT1	AF647	Abcam	ab195020	EPR3915
Hexokinase II	Dylight680	Abcam	ab228819	EPR20839
Tomm20	AF405	Abcam	ab210047	EPR15581-54
<b>Macrophage panel</b>				
CD80	FITC	BioLegend	104716	16-10A1
CD11B	PE-CF594	ThermoFisher	RM2817	M1/70.15
CD64	PE-Cy7	BioLegend	139314	X54-5/7.1
CCR2	APC	BioLegend	150604	SA203G11
MHC-II	APC-Cy7	BioLegend	107628	M5/114.15.2
CD11C	APC-R700	BD Biosciences	565872	N418
LY6C	BV605	BioLegend	128036	HK1.4
CD86	BV650	BD Biosciences	564200	GL1
CD40	BV786	BD Biosciences	740891	3/23
CD45R-B	BV750	BioLegend	103261	RA3-6B2
B7-H1	BV711	BD Biosciences	563369	MIH5
CD24	BUV737	BD Biosciences	565308	M1/69
SigF	BUV395	BD Biosciences	740280	E50-2440
IL-10	PE	BioLegend	505008	JES5-16E3
IL-1B	PerCP	ThermoFisher	46-7114-82	NJTEN3
TGF-B	BV421	BD Biosciences	565638	TW7-16B4
TNF-a	BV510	BD Biosciences	563386	MP6-XT22
<b>T cell panel</b>				
CD62L	APC-R700	BD biosciences	565159	MEL-14
TCR	APC-750	BioLegend	109246	H57-597
PD-1	BV605	BioLegend	135220	29F.1A12
CD25	BV650	BD Biosciences	564021	PC61
CD357 (GITR)	BV711	BD Biosciences	563390	DTA-1
CD45R-B	BV750	BioLegend	103261	RA3-6B2
CD3	BV785	BioLegend	100355	145-2C11
CD4	BUV395	BD Biosciences	563790	GK1.5
CD8	BUV737	BD Biosciences	612759	53-6.7
IL-17A	AF 488	BioLegend	506910	TC11-18H10.1
IL-10	PE	BioLegend	505008	JES5-16E3
Ki-67	PerCPe710	ThermoFisher	46-5698-82	SolA15
IFN-y	PE-Cy7	BioLegend	505826	XMG1.2
GATA3	PE-CF594	BD Biosciences	563510	L50-823
FOXP3	APC	ThermoFisher	17-5773-82	FJK-16s